NUCLEIC ACID MOLECULES INSERTED EXPRESSION REGULATION SEQUENCES, EXPRESSION VECTOR COMPRISING NUCLEIC ACID MOLECLUES AND PHARMACEUTICAL USE THEREOF

REFERENCE TO SEQUENCE LISTING SUBMITTED VIA EFS-WEB

[0001] This application includes an electronically submitted sequence listing in .txt format. The .txt file contains a sequence listing entitled "2021-05-06_6245-0117PUS1_ST25.txt" created on May 6, 2021 and is 46,193 bytes in size. The sequence listing contained in this .txt file is part of the specification and is hereby incorporated by reference herein in its entirety.

TECHNICAL FIELD

[0002] The present disclosure relates to a nucleic acid molecule, and more specifically, to a nucleic acid molecule enhancing expression efficiency, an expression vector comprising the nucleic acid molecule and pharmaceutical use thereof.

BACKGROUND ART

[0003] As biotechnology has been developed, various expression systems that express a gene of Interest (GOI) have been known. Among the expression systems, cell-based expression systems typically uses natural expression mechanisms of micro-organisms or eukaryotes, while other expression systems generally use purified RNA polymerases, ribosome, tRNA and ribonucleotides. In particular, proteins originated from eukaryotes perform post-translational modification such as phosphorylation, methylation and glycosylation. Since micro-organisms do not have such post-translational modification mechanisms, eukaryotic expression systems have been used in case expressing eukaryotic originated proteins.

[0004] Eukaryotic expression systems may be utilized to a gene therapy in which GOI having an open reading frame (ORF) encoding a peptide or a protein for curing various diseases is inserted in the expression systems or to a genetic vaccine in which GOI having ORF encoding a peptide or a protein such as antigens is inserted in the expression systems. The expression systems generally use nucleic acid sequences regulating transcription and/or translation of GOI so that they can express GOI efficiently within thereof. Typically, the expression systems enhance transcriptional efficiency using promoters with enhanced transcriptional efficiency, and use capping system unique to eukaryotes with regard to improving translation efficiency of GOI.

[0005] Capping system typically haw 5' cap structure of 7-methyl-guanosine (m7G) at 5' end so as to translate GOI efficiently. Translation Initiation Complex comprising translational regulation factors of eukaryotes such as eI4FA, eIF4E and eIF4G recognizes and binds to 5' cap site to form capping structure and to initiate translation for synthesizing proteins. When the capping structure is formed at the translation initiation site, the capping structure initiates protein synthesis, while it prevents mRNA degradations by nuclease actions.

[0006] It is necessary to perform in vitro transcription (IVT) process to fabricate a nucleic acid molecule with the capping structure. For example, the nucleic acid molecule with the capping structure may be fabricated by treating plasmid DNA (pDNA) with restriction enzymes so as to linearize the pDNA, translating the linearized pDNA using RNA polymerases to fabricate mRNA, and attaching m7G (5')-ppp-(5')G, i.e. regular cap analog to the mRNA at 5' end to make capped mRNA. However, such a cap analog often binds to 5' end with opposite direction, and m7G nucleotides cannot act as a cap. About one of third among the fabricated mRNA does not have methylation at the cap site, and such mRNA cannot initiate protein synthesis.

[0007] Alternatively, IVT process was performed without the cap analog, and then, cap reaction was performed using commercially available vaccinia virus capping enzymes. Besides, protein synthesis can be induced using anti-reverse cap analog (ARCA) which prevents the reverse direction reaction of the cap (ARCA-capped mRNA). It has been known that ARCA-capped mRNA can synthesize proteins as twice as the regular cap analog-capped mRNA and has much longer half-life. However, performing an artificial capping reaction (e.g. ARCA reaction) in vitro is very expensive and has low efficiency. Accordingly, it is necessary to develop an expression system that has increasing efficiencies and can be utilized as a genetic vaccine, a gene therapy, and the likes.

[0008] Immune system means a biological structure or a mechanism that detects and removes pathogens or cancer cells within an organism and thereby, protecting the organisms from various diseases. The immune system may be divided into innate immune system (inherent immune system, natural immune system) and adaptive immune system (acquired immune system).

[0009] The innate immune system is mechanism that defends a host so as to avoid an infection un-specifically and instantly responds to the pathogens without memorizing a specific pathogen. All kinds of animals and plants have innate immune system, and plants, fungi and insect have only innate immune system. In contrast, the adaptive immune system is specific to an antigen or a pathogen, and it is necessary to recognize non-self antigen through antigen-presentation process in the adaptive immune system. Accordingly, it is possible to induce a specific immune response against a specific antigen or against cells infected by the specific antigens through the adaptive immune system. Since memory cells of the adaptive immune system can recruit immune response that was performed in past, it is possible to remove the pathogen rapidly when the same pathogen infiltrate to body.

[0010] In addition, immune system can be divided into humoral immunity and cell-mediated immunity (CMI). In the humoral immunity, B lymphocyte derived from a bone-marrow recognizes antigens, differentiates to secrete antibodies consisting of glycol-protein, i.e. immunoglobulin (Ig), and then the secreted antibodies remove the infected pathogens. The CMI is an immune response that T lymphocytes derived thymus recognizes antigens so as to secrete lymphokines or kill the infected cells directly.

[0011] Vaccine antigens, which inoculate a whole pathogen or a part thereof for inducing immune responses against to the pathogens, have been used for preventions or treatments of various diseases. In this case, it is preferable to induce various immune responses caused by the vaccine antigens. Recently, sub-unit vaccines have been mainly developed in placed of early-developed attenuated live vaccines or inactivated killed vaccines because the sub-unit vaccines contain evident structures and components. How-